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Prohexadione Calcium (BX-112)

Subchronic Oral Toxicity (82-1)

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DATA EVALUATION RECORD

STUDY TYPE: 13-Week subchronic toxicity [feeding]- mouse

OPPTS Number: 870.3100

OPP Guideline Number: §82-1(b)

DP BARCODE: D246707

SUBMISSION CODE: S543930

P.C. CODE: 112600

TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): Prohexadione calcium technical (92.1% a.i.)

SYNONYMS: Cyclohexanecarboxylic acid; calcium salt of 3,5-dioxo-4-propionyl-cyclohexane-1-carboxylic acid; BX-112; KIM-112; KUH-833

CITATION: Inoue, H. (1991) Thirteen week feeding toxicity study in mice with BX-112 Technical. Biosafety Research Center, An-Pyo Center, Shizouka-ken, Japan. Laboratory Report Number 2013. May 1, 1991. MRID 44457750. Unpublished.

SPONSOR: BASF Corporation, Agricultural Products, P.O. Box 13528, Research Triangle Park, North Carolina

EXECUTIVE SUMMARY: In a subchronic oral toxicity study (MRID 44457750), prohexadione calcium (92.1% a.i.) was administered to 10 specific pathogen-free B6C3F1 mice/sex/dose at dietary concentrations of 0, 400, 2000, 10,000, or 50,000 ppm (equivalent to 0/0, 80/93, 389/454, 1986/2256, 10,244/11,916 mg/kg/day [M/F]) for 13 consecutive weeks.

All animals survived and clinical signs were not evident. Body weight gain and food efficiency in males was statistically significantly decreased ($p < 0.05$) at 400, 10,000 and 50,000 ppm in the absence of a dose response and in the presence of virtually identical numerical values at 13 weeks. Females manifested a 15 % and 9 % decrease in body weight gain and a 16 % and 11 % decrease in food efficiency at 50,000 and 400 ppm, respectively at 13 weeks. Values at 2000 and 10,000 ppm were comparable to controls for both measurements. All values occurred in the absence of statistical significance. Food consumption was generally comparable to controls. Mean corpuscular hemoglobin concentration (MCHC) was decreased ($p < 0.01$) at 13 weeks in males and females at 50,000 ppm and males at 10,000 ppm. Decreases, however, only ranged between 1-3 %. All other hematology parameters showed no statistically significant changes and only slight numerical increases indicating no support for MCHC values. Total white blood cell count (WBC) was decreased ($p < 0.01$) in males at 400, 10,000 and 50,000 ppm in the absence of

a dose response, virtually identical numerical values at these three dose levels and an apparently high control value. Values for females at all dose levels at 13 weeks were comparable to controls. The WBC count differential was also comparable to controls. Absolute organ weights for males and females were not statistically or numerically different from controls at 400 ppm and above. Organ weight to body weight changes for males and females were statistically different but were not biologically meaningful. Females showed a 7.0 % increase in kidney weight to body weight ratio at 50,000 ppm at 13 weeks in the presence of a 5 % body weight decrease. Gross pathology was not remarkable for either sex. Histopathology indicated an apparent dose response (2, 1, 3, 6, 9, control to high dose, respectively) in the renal tubules of the female kidney as seen by fatty changes graded as slight in severity. However, this same effect, graded as slight was also noted in the kidney of all males in all dose groups, including controls.

Oral toxicity NOAEL \geq 50,000 ppm (\geq 10,244/11,916 mg/kg/day [M/F])

Oral toxicity LOAEL > 50,000 ppm (>10,244/11,916 mg/kg/day [M/F])

This 13-week oral (feeding) study is classified **acceptable/nonguideline** since it was designed to select dose levels for the mouse carcinogenicity study. It does not satisfy the guideline requirement for a subchronic toxicity study in rodents since there was no clinical chemistry data provided.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: Prohexadione calcium; BX-112

Description: White/pale yellow powder

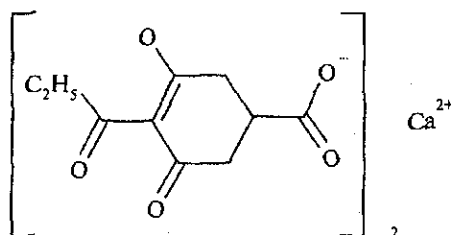
Lot #: G14-03

Purity: 92.1% a.i.

Stability of compound: Not reported

CAS #: 127277-53-6

Structure:!



2. Vehicle: Diet

3. Test animals: Species: Mouse

Strain: Specific pathogen-free (SPF), B6C3F₁ (C57BL/6XC3H)

Age and weight at treatment initiation: Approximately 5 weeks; males, 19.4-21.9 g.
females, 17.5-19.7 g

Source: JAPAN SLC, Inc. (3371-8, Koto-cho, Hamamatsu-shi, Shizuoka, Japan)

Housing: Individually housed in wire mesh cages

Diet (basal): NIH Open Formula Rat and Mouse Ration (Oriental Yeast Co., Ltd., Chuo-Ku, Tokyo), sterilized by gamma radiation, ad libitum

Water: Tap water, ad libitum

Environmental conditions:

Temperature: 22-24°C

Humidity: 50-60%

Air Changes: 20/hour

Photoperiod: 12-hour light/dark cycle

Acclimation period: 8 days

B. STUDY DESIGN:

1. In life dates - start: 06/21/88 end: 09/21/88
2. Animal assignment - Mice were assigned to the test groups in Table 1 using a randomized procedure (not further described).

Table 1. Study design^a

Test Group	Conc. in Diet (ppm)	Achieved Mean Dose (mg/kg/day) ^b [M/F]	Animals Assigned	
			Male	Female
Control	0	0	10	10
Low	400	80/93	10	10
Mid	2000	389/454	10	10
High	10,000	1986/2256	10	10
Super high	50,000	10,244/11,916	10	10

- a Dose selection was based on the knowledge that prohexadione calcium is a weak poison; the 50,000 ppm dose was chosen as the maximum practical dietary dosage and the lower doses were fixed at 1/5, 1/25, and 1/125 of the 50,000 ppm dose.
- b Mean daily test substance intake values provided on page 21 of the study report.

3. Treatment preparation and dosing - On a weekly basis, the test substance was mixed with basal diet; storage conditions were not provided. For concentration analyses, two samples of prepared food mix at concentrations of 336, 1680, 8400, and 42,000 ppm were analyzed at unspecified intervals during the 13-week study; samples were examined in duplicate. Homogeneity and stability analyses were not conducted; however, in a subchronic oral toxicity study reviewed with the current submission (MRID 44457749), prohexadione calcium was stable in the diet (NIH Open Formula Rat and Mouse Ration) when stored at room temperature for up to 16 days (86.9-88.6% of nominal) at concentrations of 30,000 and 50,000 mg/kg.

Results

Concentration analyses (mean % of nominal): 336 ppm, 86.9%; 1680 ppm, 83.9%; 8400 ppm, 87.6%; and 42,000 ppm, 90.2%.

The analytical data indicated that the variance between nominal and actual dosage to the animals was acceptable.

4. Statistics - Body weights, food consumption, food efficiencies, hematological evaluations, and absolute and relative organ weights were analyzed for homogeneity of variances using Bartlett's test. If the variances followed a normal distribution, the data were analyzed using an analysis of variance followed by Dunnett's or Duncan's t-tests. If variances were heterogeneous, the data were analyzed using Kruskal-Wallis and Dunnett's tests (for non-parametric data).

C. METHODS

1. Observations - Animals were observed for mortality and clinical signs of toxicity at least twice daily; additional observations were recorded on Sundays if dead or moribund animals were observed.
2. Body weight - Individual body weights were recorded pre-test and once weekly during the 13-week study.
3. Food and compound intake/water consumption - Mean food consumption (g/week) was determined at weekly intervals. Food efficiency (%) and mean compound intake (mg/kg/day) were calculated from body weight and food consumption data.
4. Ophthalmoscopic examination - Ophthalmoscopic examinations were not performed.
5. Blood - At week 13, all surviving mice were fasted for 16 hours and blood was collected from the abdominal aorta under ether anesthesia. The checked (X) parameters were examined in all samples analyzed.

a. Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpuscular HGB concentration (MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)
X	Platelet count*		Absolute lymphocytes
	Blood clotting measurements*		Absolute segmented neutrophils
	(Thromboplastin time)		Erythrocyte morphology
	(Clotting time)		
	(Prothrombin time)		

* Required for subchronic toxicity studies.

b. Clinical chemistry - Clinical chemistry analyses were not performed.

6. Urinalysis - Urinalysis examinations were not performed.
7. Sacrifice and pathology - After 13 weeks of treatment, all surviving animals were sacrificed (not further described) and subjected to a gross pathological examination. The weights of the brain, heart, liver, kidneys, spleen, adrenals, and testes/ovaries were recorded. All gross lesions, liver, kidneys, spleen, heart, adrenals, and testes/ovaries were examined microscopically.

II. RESULTS

A. Observations

1. Mortality - All animals survived the 13-week study.
2. Clinical signs - No clinical signs of toxicity were observed during the study.

B. Body weight - No significant differences in body weights were observed between control and treated animals throughout 12 weeks of treatment. At 13 weeks, decreases ($p \leq 0.05$) in body weights were observed in the 400 (16%) and 50,000 ppm (16%) males when compared to controls. Similarly, decreases ($p \leq 0.05$) in overall (0-13 weeks) body weight gain were observed in the 400 (118%) and 50,000 ppm (117%) males. These decreases, however, were not dose-dependent, and therefore, were not considered to be treatment-related. Females manifested a 15 % and a 9 % decrease in body weight gain at 50,000 and 400 ppm at 13 weeks. Values for females at 2000 and 10,000 ppm were comparable to controls. Values occurred in the absence of statistical significance. At 13 weeks, mean body weights ranged from 29.7-31.7 g for males and 26.3-27.8 g for females.

C. Food consumption and compound intake

1. Food consumption - No treatment-related effects on mean food consumption (g/week) or food efficiency were observed during the 13-week treatment period. Decreases ($p \leq 0.05$ or 0.01) in overall (0 to 13 weeks) food efficiency were observed in 400, 10,000, and 50,000 males when compared to controls (121%); however, the differences were slight and not dose-dependent, and therefore, not considered to be treatment-related. Females showed a 16 % and an 11 % decrease in food efficiency at 50,000 and 400 ppm at 13 weeks. Values at 200 and 10,000 ppm were comparable to controls for both dose levels.
2. Compound consumption - The achieved mean dosages based on nominal dietary concentrations, actual body weights, and actual food consumption are shown in Table 1.
3. Water consumption - Water consumption was not measured.

D. Ophthalmoscopic examination - Ophthalmoscopic examinations were not conducted.

E. Blood work

1. Hematology - Mean corpuscular hemoglobin concentration (MCHC) was decreased ($p < 0.01$) at 13 weeks in males and females at 50,000 ppm and in males at 10,000 ppm. Decreases, however, only ranged between 1-3 %. All other hematology parameters showed no statistically significant changes and only slight numerical increases indicating no support for MCHC values being treatment related. Total white blood cell (WBC)

count was decreased ($p < 0.01$) in males at 400 ppm and above in the absence of a dose response, with virtually identical numerical values at these three dose levels and an apparently high control value. Values for females at all doses levels at 13 weeks were comparable to controls. The WBC count differential was also comparable to controls.

2. Clinical chemistry - Data not submitted.

F. Sacrifice and pathology

1. Organ weight - No treatment-related differences in absolute or relative (to body) organ weights were noted between the treatment and control groups. Findings ($p \leq 0.05$) included: increases in absolute (16%) and relative (16%) liver weights of 2000 females and increases in relative ovary weights of 400 and 2000 ppm females (132 and 104%, respectively). These increases were not dose-dependent and not considered to be treatment-related. Increases (17%) in relative kidney weights in the 50,000 ppm females were not statistically significant and were correlated with the lower body weights of this group.
2. Gross pathology - No treatment-related gross postmortem findings were observed in any of the treatment groups. All abnormalities occurred randomly and sporadically in all study groups.
3. Microscopic pathology

a) Non-neoplastic - Fatty changes of the renal tubular epithelium were observed in 2/10, 1/10, 3/10, 6/10, and 9/10 females from the control, 400, 2000, 10,000, and 50,000 ppm groups, respectively. Although this effect was observed in two control animals, the increase in incidence may be suggestive of a treatment-related response at the 10,000 and 50,000 ppm levels. However, this same effect, graded as slight was also noted in the kidney of all males in all dose groups including controls. Additional findings, such as, liver granulation and spindle cell hyperplasia of the adrenal, were not considered to be the result of treatment since they were either observed in control animals, did not follow a dose-response pattern, or were sporadically observed.

b) Neoplastic - No neoplastic tissue was observed in the treated mice.

III. DISCUSSION

- A. Investigator's conclusions - The study author concluded that the LOAEL for prohexadione calcium (BX-112) is 50,000 ppm (equivalent to 10,244/11,916 mg/kg/day M/F) based on decreased body weights of male mice and histological changes in kidneys of female mice. The resultant NOAEL is 10,000 ppm (equivalent to 1986/2256 mg/kg/day M/F).

- B. Reviewer's Discussion - In a subchronic oral toxicity study, prohexadione calcium (BX-112) was administered for 13 weeks to 10 specific pathogen-free B6C3F1 mice/sex/dose at dietary concentrations of 0, 400, 2000, 10,000, or 50,000 ppm (equivalent to 0/0, 80/93, 389/454, 1986/2256, 10,244/11,916 mg/kg/day [M/F]). The analytical data indicated that the variance between nominal and actual dosage to the study animals was acceptable.

No animals died during the study and no treatment-related effects on clinical signs, body weight, food consumption, food efficiency, organ weights, or gross pathology were observed. Clinical chemistry analyses of blood and ophthalmoscopic examinations were not conducted.

Hematology analyses resulted in differences ($p \leq 0.01$) in 10,000 and 50,000 ppm males and 50,000 ppm females including: decreased mean corpuscular hemoglobin concentrations (11-3%), and decreased white blood cell counts (142-47%). However, all other hematology parameters showed no statistically significant changes and only slight numerical increases indicating no support for MCHC values. Total WBC counts were decreased ($p < 0.01$) in males at 400 ppm and above; in the absence of a dose response, virtually identical numerical values, and an apparently high control value. Values for females at all dose levels at 13 weeks were similar to controls. The WBC count was also comparable to controls.

Microscopic examinations were limited to all gross lesions, liver, kidneys, spleen, heart, adrenals, and testes/ovaries. Treatment-related microscopic abnormalities were limited to dose-related increases in the incidence of fatty changes of the renal tubular epithelium in 10,000 and 50,000 ppm females; this effect, graded as slight for all observations was observed in 2/10, 1/10, 3/10, 6/10, and 9/10 females from the control, 400, 2000, 10,000, and 50,000 ppm groups, respectively. However, it is also noted that all males in all groups were observed with fatty changes of the renal tubular epithelium with a severity grade of slight.

No neoplastic tissue was observed in the treated or control mice.

Oral toxicity NOAEL \geq 50,000 ppm (\geq 10,244/11,916 mg/kg/day [M/F])

Oral toxicity LOAEL $>$ 50,000 ppm ($>$ 10,244/11,916 mg/kg/day [M/F])

This 13-week oral (feeding) study is classified **acceptable/nonguideline** since it was designed to select dose levels for the mouse carcinogenicity study. It does not satisfy the guideline requirement for a subchronic toxicity study in rodents since there was no clinical chemistry data provided.

IV. STUDY DEFICIENCIES

The following deficiencies were noted, but will not affect the conclusions of this report:

- clinical chemistry parameters were not measured; these clinical chemistry parameters

- must be measured in a subchronic study
- blood clotting measurements were not conducted
- ophthalmoscopic examinations were not performed
- many required organs were not examined microscopically
- homogeneity analyses were not conducted, however the concentration of the compound in the diet as well as homogeneity and stability have been adequately presented either directly or indirectly by the registrant.